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13. Abstract (Maximum 200 Words) <i>(abstract should contain no proprietary or confidential information)</i> I have proposed to identify peptides that bind to the vasculature of prostate cancers by using a technique developed in our laboratory called " <i>in vivo</i> phage display". These peptides would have the potential to be used in drug targeting. The tumor-specific peptides will then be used to isolate their vasculature-specific receptors. The receptors could be specific markers for the vasculature of prostate cancers and lead to a greater understanding of what kinds of vascular surface proteins are up-regulated during prostate carcinogenesis. Because of the difficulty in the experimental procedure, I took an alternative approach, mRNA profiling by GeneChip or cDNA microarray. To this end, I have accomplished the following goals: 1. Established GeneChip and cDNA Microarray systems in our laboratory. 2. Established an <i>in vitro</i> transcription method to amplify limiting amount of RNA that can be obtained from mice. 3. Improved endothelial cell purification procedures by adopting magnetic beads to increase yield. 4. Screened endothelial cell specific markers from lung and kidney.			
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Table of Contents

Cover.....	1
SF 298.....	2
Table of Contents.....	3
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	5
Reportable Outcomes.....	6
Conclusions.....	6

Introduction:

I have proposed to identify peptides that bind to the vasculature of prostate cancers by using a technique developed in our laboratory called "*in vivo* phage display". These peptides would have the potential to be used in drug targeting. The tumor-specific peptides will then be used to isolate their vasculature-specific receptors. The receptors could be specific markers for the vasculature of prostate cancers and lead to a greater understanding of what kinds of vascular surface proteins are up-regulated during prostate carcinogenesis.

During the first stage, I had difficulty in the *in vivo* phage display approach because of the composition and size of the tumors grown in nude mice, so I adopted an alternative approach: the direct isolation of endothelial cells from various organs and tumors followed by a comparison of their mRNA profiles as determined by GeneChip and cDNA Microarray technologies. By identifying overexpressed genes in the endothelial cells of different organs and tumors, potential targets for differential drug delivery will be revealed.

Because GeneChip and cDNA Microarray technologies are relative new, I have established three inducible cell lines to test the system. I succeeded in establishing the system in our laboratory and proceeded to screen for organ and tumor specific endothelial cell markers. Present data indicate this approach could be fruitful. I am in the process of making findings with this approach.

Body:

Established GeneChip and cDNA Microarray systems in our laboratory

GeneChip and cDNA Microarray are exciting new technologies that promise to speed up new discoveries in this genomic era. However, the tedious steps of setting up the system and the magnitude of the data sets prevents guaranteed success in every laboratory. In order to successfully establish these systems in our laboratory, I have established three inducible cell lines that I can use to test the system. The inducible nature of this cell line system is perfect to verify the complex GeneChip and cDNA Microarray systems. I was able to show that an induced R-Ras gene is faithfully detected in both GeneChips and cDNA Microarray systems. Along with R-Ras gene, there are several genes that are detected as either suppressed or induced upon R-Ras's induction. Some of them are due to the Ecdysone that I used to induce R-Ras, and the others are affected by the over-expression of R-Ras. The results are confirmed by northern blot analysis. Although both methods are quite expensive, multiple experiments are absolutely required to avoid false positives and false negatives, which arise because of the nature of quantity of data.

Improved endothelial cell purification procedures

Tie-2/LacZ mice specifically express the beta-galactosidase gene in endothelial cells, and I have established a cell sorting method to purify endothelial cells from these mice. While the purity is very high, it takes a rather long time to collect substantial amount of cells for mRNA profiling study. I have improved the method by using magnetic beads to replace cell sorting step and can obtain larger amount of cells in a shorter time at the expense of slight decrease in purity. By using this method, I was able to collect more endothelial cells and also to do several preparations at the same time.

Screen endothelial cell specific markers

With the endothelial cells that I purified from Tie2/LacZ mice and a cDNA Microarray system, I compared the expression profiles of non-endothelial cells and endothelial cells in two different organs -- lung and kidney. By using IVT-amplified samples, I was able to obtain a list of genes that were expressed at higher levels in endothelial cells than non-endothelial cells. Among them are Endoglin, ICAM2, Flt1, Ly6c, MHC class I heavy chain precursor (H-2K(b)), Calcitonin activity modifying protein 2 (Ramp2), Scavenger receptor class B1, VE-cadherin, and Flt4. Most genes of this list have been shown to be endothelial cell specific and are in the published literature. It shows that my system is capable of detecting endothelial cell specific genes although the starting materials are limiting and need to be amplified. Besides the genes shown above, I have also identify seven, unreported EST clones, and am in the process of using *in situ* hybridization to confirm these findings.

Purified endothelial cells from Tie-2/LacZ x TRAMP mice

The final goal is to identify prostate cancer-specific endothelial cell markers. To this end, I have crossed Tie-2/LacZ mice with TRAMP mice. The male offspring developed prostate cancer as expected, and beta-galactosidase is expressed in the prostate cancer's endothelia. I have purified these endothelial cells, and am currently verifying the purity of the preparation. I will profile the mRNA expression levels once I have verified a satisfactory purity.

List of key research accomplishments:

1. Established GeneChip and cDNA Microarray systems in our laboratory.
2. Established an *in vitro* transcription method to amplify limiting amount of RNA that can be obtained from mice.
3. Improved endothelial cell purification procedures by adopting magnetic beads to increase yield.
4. Screened endothelial cell specific markers from lung and kidney

Reportable Outcomes:

Seven EST clones are detected first time to be endothelial cell specific in mouse lung and kidney.

Conclusions:

I have established cDNA microarray method that could detect prostate cancer specific endothelial cell markers. The method has been tested in the inducible cell line system. I also used this method to identify multiple pan-endothelial cell markers. I will continue using this method to identify prostate cancer specific endothelial cell markers.